

Regular Article

Activation of rice plant growth against *Rhizoctonia solani* using *Pseudomonas fluorescens*, *Trichoderma* and Salicylic Acid

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From rhizosphere soil of organic farming fields of major vegetable growing tracts of Kerala, *Pseudomonas fluorescens* and *Trichoderma* sp. were isolated. The antagonistic activity of isolates was observed against *Rhizoctonia solani*. Biological control and hormonal inducers represents an interesting strategy against pathogen especially when applied together. Rice plants infected with *R. solani* were treated with Biocontrol agents along with arbuscular mycorrhizal (AM) fungi and/or sprayed with hormonal inducers (Salicylic acid). Plants were harvested on 14 and 28 days after pathogen infection growth rate of the plant was measured. Plants treated with biocontrol agents alone showed moderate growth. Likewise VAM alone treated plants showed very good result whereas combination of VAM and salicylic acid did not show considerable growth response. Biocontrol agents *Trichoderma* and *P. fluorescens* when applied along with salicylic acid showed appraisable increase in the biometric parameters in rice against *R. solani* and decrease the percentage of rate of infection compared with control and other treatments.

Key words: *Rhizoctonia solani*; rice; *P. fluorescens* and *Trichoderma* sp.; Salicylic acid and plant growth response.

Soil borne pathogens are complex not only in their behavioral pattern but also in their biochemical constituents. Hence, it is not very easy to control these pathogens. *Rhizoctonia solani* is a plant pathogenic fungus with a wide host range and worldwide distribution. It is the major fungus responsible for damping-off, black spot and root rot diseases (Neha and Dawande, 2010). With most vegetables, no effective fungicides are available against *Rhizoctonia* diseases. Intensified use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans & environment also in the buildup of resistance of pathogens (Amel *et al.*, 2010). In order to tackle these national and global problems, alternatives of

chemical control are investigated by the use of antagonistic microbes (Deacon, 1991).

Biological control means control of disease through any living microorganism. Biocontrol of soil-borne plant pathogens affecting agricultural plants can be controlled by the use of species of *Trichoderma*, *Aspergillus*, *Trichothecium* and *Epicoccum* in India. There are some antagonistic bacteria like *Bacillus subtilis*, *Enterobacter aerogenes*, *Pseudomonas fluorescens*, *Streptomyces* sp. and *Actinomycetes* in disease control (Chet, 1990). *Trichoderma* sp. has been widely studied as potential biocontrol agents for controlling many plant pathogens. *Trichoderma* sp. has been known to control plant diseases biologically for more than 70

years (Gary *et al.*, 2003). *Pseudomonas* sp. is ubiquitous in agricultural soils, well adapted to growing in the rhizosphere. *Pseudomonas* possesses many traits that make them well suited as biocontrol and growth-promoting agents (Weller, 1988; David, 2007). This study was undertaken to develop the most effective biocontrol treatment for the plant pathogenic fungi *Rhizoctonia solani* by combining the use of antagonistic microorganisms along with the hormonal inducer salicylic acid.

Materials and Methods

In the present study, the biocontrol agents namely *Pseudomonas fluorescens* and *Trichoderma* sp. isolated from rhizosphere soil of organic farming area were used.

Isolation of antagonistic strains

Rhizosphere soil of organic farming field collected from the major vegetable growing tracts of Kerala was used for isolation. Soil samples were collected during October to November 2010 and isolation was done following dilution plate technique. Bacterial colonies showing the characteristic fluorescence in King's medium B (KMB) were picked up, purified, maintained on KMB slants. Fungal colonies with white mycelium, which later changed into different shades of green on Potato Dextrose Agar (PDA) medium, were examined, purified, transferred to PDA slants.

Source of pathogenic fungi and rice seedlings

Pure culture of plant pathogenic fungi *Rhizoctonia solani* was procured from Kerala Agricultural University. Meanwhile, rice seedlings, were obtained from Vegetable Crops Research Dept., Thanjavur Rice Research Center, Thanjavur.

Preparation of VAM

Glomus fasciculatum - *Glomus fasciculatum* was used to make Vesicular arbuscular mycorrhizal fungi obtained from Tamil Nadu Agricultural University, Coimbatore. For this, corn seeds were

soaked in water for 5-6 hrs. The potting mix consisted of soil and organic mixture in the ratio 2:1 and 5kg each of potting mix were filled in the pots. In the surface soil 250gm VAM fungi (*Glomus fasciculatum*) was inoculated and mixed evenly. The soaked corn seeds were then seeded in 1cm depth and it was watered daily. When the corn plants have acquired sufficient growth, VAM was harvested by removing the plants from the pot. The roots of the plants were also cut into pieces and mixed with the VAM. This is then used for the soil treatment.

Biocontrol activity test

Biocontrol of plant pathogen *R. solani* by *Trichoderma* sp. and *P. fluorescens* were tested by coculturing test organism on malt extract agar plate on same culture plate and growth of were examined after 3 – 7 days.

Preparation of *Pseudomonas fluorescens*, *Trichoderma* and *Rhizoctonia solani* for seedling treatment

Preparation of *Rhizoctonia solani*

Rhizoctonia solani of rice was inoculated 400ml of Potato Dextrose Broth and incubated at room temperature on a rotary shaker for 7 days.

Preparation of isolates

200ml of Kings B broth was prepared and it was inoculated with a loop full culture of *P. fluorescens*, incubated at room temperature on a rotary shaker for 5 days. 200ml of Potato Dextrose broth was prepared, inoculated with a loop full culture of *Trichoderma*. It was incubated at room temperature on a rotary shaker for 7 days.

Efficacy of biocontrol agents against *Rhizoctonia solani* of rice under field condition

Inoculation of pathogen

7 days old culture of *Rhizoctonia solani* in PDB was used for inoculation. 12 ml of culture was inoculated for each treatment by root prick method by giving fine pricks in the rooting area of the plant.

Seedling treatment

Germinated paddy seedlings were dipped in *Trichoderma* sp. and *P. fluorescens* suspension having 10^8 CFU/ml for half an hour and planted in the respective plots. In V1 and V2, 5% of VAM was added as inoculum for each plot (250gm/ 5kg soil).

Setting of experimental plots

7 plots each having an approximate area of one square feet and 15cm depth was made for rice and a plastic sheet was placed in the ditch over which 5kg of soil was spread.

Application of salicylic acid

200 μ l salicylic acid was sprayed to T2, P2 and V2 four times. First spraying was done 2 days before the inoculation of pathogen. Second spraying was done in the same day of inoculation of pathogen. The spraying was repeated in 9 days interval for two times. The treatments were as follows (Samia and Khallal, 2007)

C - Control plants inoculated with pathogen *R. solani*.

T1 - Plants treated with *Trichoderma* 7 days before inoculation of pathogen.

P1 - Plants treated with *P. fluorescens* 7 days before inoculation of pathogen.

V1 - Plants treated with vesicular arbuscular mycorrhizal fungi 7 days before inoculation of pathogen.

T2 - Plants treated with *Trichoderma* 7 days before inoculation of pathogen and treated with salicylic acid at regular intervals.

P2 - Plants treated with *Pseudomonas fluorescens* 7 days before inoculation of pathogen and treated with salicylic acid at regular intervals.

V2 - Plants treated with vesicular arbuscular mycorrhizal fungi 7 days before inoculation of pathogen and treated with salicylic acid at regular intervals.

All the treatments and control plant were compared by observing the rate of infection and other biometric parameters namely shoot, root length, collar diameter,

number of leaves on 14th day and 28th day after the inoculation of pathogen.

Result

Isolation and identification of microorganisms

Rhizosphere soil of organic farming fields of major vegetable growing tracts of Kerala was used for isolation. Bacterial colonies appeared on the surface of Kings B medium was identified as *Pseudomonas fluorescens* based on morphological and biochemical reactions. Well isolated colonies of were observed in Rose Bengal agar after 5 days. Initially the mycelia were found to be whitish. At maturity the fungal colonies was seen as dark green coloured colony. By staining using lacto phenol cotton blue, the fungal growth was identified as *Trichoderma* sp.

Invitro the antagonistic activity of isolates

The antagonistic property of *Trichoderma* sp. and *P. fluorescens* was observed against *Rhizoctonia solani*. On coculturing, growth of only antagonists was observed in respective media while growth of *R. solani* was suppressed. Thus the study revealed effectivity of isolates and their potential against plant pathogens.

Field application of biocontrol agents against *Rhizoctonia solani* of rice

The efficacy of biocontrol agents *P. fluorescens*, *Trichoderma* sp. and VAM fungi was applied on rice plants alone and along with hormonal inducer salicylic acid against *R. solani* was measured. Plants were harvested on 14 and 28 days after pathogen infection and growth rate of these treatments associated with pathogens infection was monitored.

Among the treatments in which biocontrol agents applied without the application of salicylic acid, VAM was found to be more effective (Table 1 & 2). All the salicylic acid treated plants showed an increase in biometric parameters. Infection rate was also found to be decreased in salicylic acid treated plants. Salicylic acid when applied along with biocontrol agents

Trichoderma sp. and *P. fluorescens* showed increased plant growth and decrease the percentage of rate of infection. VAM alone treated plants showed very good result

where as combination of VAM and salicylic acid did not show considerable increase in growth and reduction in percentage of infection (Plate 1).

Table 1. Efficacy of biocontrol agents against *Rhizoctonia solani* in rice (14 days)

Treat ment	Shoot length	Root length	No. of leaves	No. of roots	No. of infected leaves	% of infection	Colar diameter (cm)	Fresh weight (gm)	Dry weight (gm)
C	11.6	5.12	3.8	9.8	2.8	73.2	0.54	0.14	0.05
T ₁	12.86	6.74	4.6	10.2	1.6	29.6	0.7	0.23	0.08
P ₁	12.92	5.44	3.8	10	1.2	30	0.6	0.17	0.05
V ₁	13.74	5.66	4.6	9.8	1	22	0.94	0.26	0.08
T ₂	16.4	7.34	5	12.4	1.6	23.6	0.8	0.32	0.09
P ₂	15.76	7.94	5.4	11.8	1.4	25.2	0.8	0.28	0.07
V ₂	13.26	8.46	5	12.4	1	21	1.04	0.36	0.10

Table 2. Efficacy of biocontrol agents against *Rhizoctonia solani* in rice (28 days)

Treat-ment	Shoot length	Root length	No. of leaves	No. of roots	No. of infected leaves	% of infection	Colar diameter (cm)	Fresh weight (gm)	Dry weight (gm)
C	13.8	6.02	3.4	9.2	3	90	0.7	0.35	0.13
T ₁	17.12	7.78	6.2	13.2	2.2	38.6	1.42	0.6	0.18
P ₁	16.64	7.5	5.8	14	2.2	37.8	1.5	0.61	0.19
V ₁	19.46	8.84	6.4	14.4	2	31.8	1.46	0.73	0.18
T ₂	18.78	8.08	6.8	15.2	2	29.8	1.44	0.88	0.21
P ₂	18.84	8.8	6.6	13.6	2	30.6	1.56	0.88	0.21
V ₂	18.9	9.82	7	14	2	29	1.58	0.94	0.21



Plate 1. Induction of plant growth treated with biocontrol agents against *Rhizoctonia solani* in rice

Discussion

The present study was carried out to develop an effective biocontrol agent against *Rhizoctonia solani* of rice. For this, biocontrol agents *Pseudomonas fluorescens* and *Trichoderma* sp. was isolated from rhizosphere soil organic farming field. Early studies had revealed that species of *Pseudomonas* and *Trichoderma* had biocontrol activity against plant pathogens (Weller, 1988 and Neha and Dawande, 2010).

Usually the biocontrol agents are used against the pathogen in order to reduce the disease producing activities thereby reducing crop loss (Chet, 1990). Biometric parameters in rice can be increased using biocontrol agents when applied along with salicylic acid. The percentage of rate of infection was also decreased. VAM treated plants also showed better growth stimulation but less effective when applied along with salicylic acid. Similarly application of bioagent arbuscular mycorrhiza (AM) and hormonal inducers (JA or SA) enhanced shoot and root growth of *Fusarium* infected plants, especially when applied together. This study was reported by Samia and Khallai (2007).

AM fungi greatly increase host tolerance against pathogen attack by compensating for the loss of root biomass or function caused by pathogen (Morgan *et al.*, 2005). A synergistic effect of JA and AM inoculation on shoot and root growth were found in *Allium sativum* (Regvar *et al.*, 1996). The increase in shoot and root length especially treated with AM fungi and SA may be related to the action of cellulose and pectinases on host cell walls which would decrease the level of lignin cell wall- bound phenolic compounds (Ikegawa *et al.*, 1996). From the above it has been concluded that the biocontrol agents *Pseudomonas fluorescens* and *Trichoderma* sp. isolated from rhizosphere of organic farming area are effective against *Rhizoctonia solani*. Development of biological control product based on these strains is needs further research on repeated trail in field and to

study the best formulation to ensure success of the control mechanism of the isolated rhizosphere organisms.

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